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Dirofilaria immitis infection in a Dachshund dog: Diagnosis and Treatment

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Abstract

Dirofilaria immitis is a filariid worm which typically lives as adult in the right ventricle of the heart and the pulmonary arteries of the dog, causing the canine heartworm disease. Therapy of *canine dirofilariosis* due to *Dirofilaria immitis* is indicated for dogs suffering from clinical signs of the disease (such as chronic cough). The present paper describes diagnostic and treatment features of a *D. immitis* affected dog in Ahvaz district, Southwest of Iran. The dog had coughing, tachypnea, labored breathing, panting, hind limb edema, weakness, and exercise intolerance, at the time of referral. Auscultation revealed grade III/VI systolic murmur over the left apex of the heart. Giemsa stained blood smears containing microfilaria was morphologically identified as *D. immitis*. CBC revealed an inflammatory leukogram and mild anemia. Combined therapy with ivermectin (440 µg/kg as single dose), levamisol (10 mg/kg q 24 h for 10 days) and aspirin (10 mg/kg q 12 h for 10 days), during 2 stages, was effective. The number of microfilaria dropped from 1250±50/ml blood pre-treatment to 150±10/ml (following the first treatment stage, after 10 days) and reached 0 a day after the second treatment. The animal remained negative regarding *D. immitis* microfilaremia during a follow-up period of 180 days. This record confirms the efficacy of ivermectin with levamisol and aspirin in the treatment of adult heartworm infection in a dog.

Keywords: *Dirofilaria immitis*, Dog, Ivermectin, Levamisol, Aspirin

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Introduction

Heartworm disease is caused by *Dirofilaria immitis*, a parasitic worm that lives as adult in the right side of the dog's heart and large blood vessels leading to the lungs. Heartworms can cause serious cardiopulmonary disease in their hosts. *Canine heartworm* has become widespread in many parts of the world, and its range continues to expand. Wildlife reservoirs play a role in perpetuation and transmission of this parasite to dogs (Greene, 2006, Lee *et al.,* 2010). Although adult *Dirofilaria immitis* affects dogs, cats, foxes and other wild mammal, larvae have been reported sporadically in humans from various parts of the world. Most infected dogs do not show any symptoms of the disease for a long time, months or years (Dillon *et al.,* 1995). Signs of the disease develop gradually and may begin with a chronic cough. Sudden death rarely occurs and usually it happens following respiratory distress (Venco, 2004). Cases of ocular dirofilariosis due to *D. immitis* have been previously recorded in Australia, the United States and Europe (Dantas-Torres *et al.,* 2009). Diagnosis of heartworm infection can be made in dogs by blood test detecting circulating microfilaria or adult antigens but further diagnostic procedures are usually required to determine the severity of disease and the best treatment (Knight, 1995). Tests designed to detect heartworm adult antigens based on ELISA or colloidal gold staining techniques are considered highly specific and cross reactivity with other dog's parasites (i.e. *D. repens*, *Dipetalonema* sp.) does not occur (Greene, 2006, Schnyder *et al.,* 2011). It has been said that the treatment of heartworm infection is difficult. Symptomatic therapy includes drugs and measures that can improve cardiopulmonary circulation and lung inflation. Restriction of exercise and, in selected cases, cage rest seems to be the most important measure to improve cardiopulmonary circulation and to reduce pulmonary hypertension (Dillon *et al.,* 1995). Anti-inflammatory doses of glucocorticosteroid (prednisolone 2 mg/kg s.i.d. for four or five days) given at diminishing rate can control pulmonary inflammation and thromboembolism. Diuretics (furosemide 1 mg/kg b.i.d.) are useful to reduce fluid effusions caused by right congestive heart failure. Digoxin may be administered only to control atrial fibrillation. The organical arsenical melarsomine dihydocloride is the only available compound to be used in the adulticide heartworm therapy in dogs. Two intramuscular injections of 2.5 mg/kg 24 h apart is the standard regimen (Greene, 2006). In fact, one administration of melarsomine at dose of 2.5 mg/kg kills about 90% of male worms and 10% of female worms resulting therefore in 50% reduction of the worm burden (Di Sacco and Vezzoni, 1992, Vezzoni et al., 1992). It is now known that macrocyclic lactones have evidence of resistance in *D. immitis* (Bourguinat *et al.,* 2011). Spontaneous pneumothorax has been reported in a dog secondary to *D. immitis* infection (Oliveira *et al.,* 2010). Experimental studies have shown ivermectin to have partial adulticidal properties when used continuously for at least 16 months at preventative doses (6-12 mcg/kg/month) and 100% adulticidal efficacy if administered continuously for over 30 months (McCall *et al.,* 2001, Venco *et al.,* 2004). To the authors' knowledge, a few published reports have described treatment and clinical management of canine *D. immitis* infection. This paper describes diagnostic and treatment features of a naturally infection of *D. immitis*, in Ahvaz district, Southwest of Iran.

Case history

A 7.5-year-old female spayed Dachshund was referred to the Veterinary Hospital in Ahvaz, Southwest of Iran. On physical examination, the dog showed coughing, tachypnea, labored breathing, panting, hind limb edema, weakness, and exercise intolerance. Respiratory rate was increased of 46 breaths/min and a normal heart rate of 108 beats/min. Auscultation revealed grade III/VI

systolic murmur over the left apex of the heart. Blood sample was examined after concentration (Knott test) for the presence of microfilaria. A microfilarial count was performed from venous blood in EDTA for five times. The blood was mixed in the tube for 5 min by placing on a tube roller, in order to allow equal dispersion of the microfilaria. Thin blood smears were made from 3 μl of blood measured by a micropippete. Smears were stained with Giemsa. The serum sample was separated in vacuum blood tubes by centrifugation at 1000 rpm for 5 min. Serum was examined with a commercial Rapid *D. immitis* Ag Test kit (Cat No: RB21-03) by Immunochromatography assay (ICA) (Manufactured by Anigen, Animal genetics, Inc., Korea). This kit is a chromatographic immunoassay for the qualitative detection of *D. immitis* antigen in whole blood, plasma or serum. Sensitivity and specificity of these kits were 95.8 and 99.7%, respectively (according to the manufacturer's instructions). Treatment of the edema was initiated with 2 mg/kg of furosemide and 1 mg/kg of prednisone once a day. Ultrasonographic examination of the heart and abdomen and an electrocardiogram (ECG) were performed. Combined therapy with ivermectin (440 microgram/kg as single dose), levamisol (10 mg/kg q 24 h for 10 days) and aspirin (10 mg/kg q 12 h for 10 days) were performed in the Dachshund dog. Prothrombin time (PT) and an activated partial thromboplastin time (APTT) were evaluated for a possible coagulopathy.

The Anigen Rapid *D. immitis* Ag test kits were used in the present study. Interpretation of test results is at 5-10 min. If a color band appears in the left section of the result window, it means that the test is working properly. This is the control band. If another colored band is appeared in the right section of the result window, it would be the test band. The presence of two colored bands (T and C) indicates a positive result within the result window (according to the manufacturer's instructions).

Results

Microscopical examination of the blood smear revealed microfilaremia with a filarial species. Giemsa stained blood smears was morphologically identified as *D. immitis*. A modified Knott's test was used for detection of microfilaria (Fig. 1). Serology for *D. immitis* antigen (Anigen Rapid *D. immitis* Ag Test Kit) showed positive reaction (Fig. 2). The number of microfilaria dropped from 1250±50/ml blood pre-treatment to 150±10/ml (following the first treatment, after 10 days) and reached 0, one day after the second treatment. A modified Knott's test was negative 24 h post the second treatment when the dog was discharged from hospital. No microfilaria was found in blood. The dog remained negative for *D. immitis* microfilaremia during a follow-up period of 180 days. Prothrombin time and an activated partial thromboplastin time were within reference range. Six months after the initial treatment, the dog was admitted again. A repeated microfilaria count and modified Knott's test were negative. Thoracic radiograph was normal. There was no evidence of *D. immitis* worms in related organs. No abnormalities were detected on complete blood count, serum biochemistry panel, thoracic radiographs, abdominal ultrasound and electrocardiogram (Fig. 3).

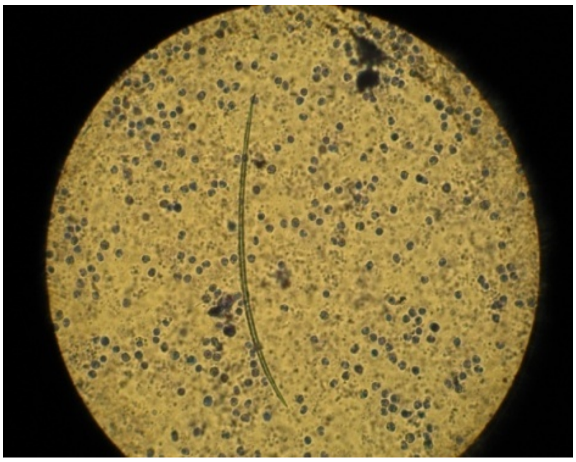


Figure1: Microfilaria of *Dirofilaria immitis* by Knott test

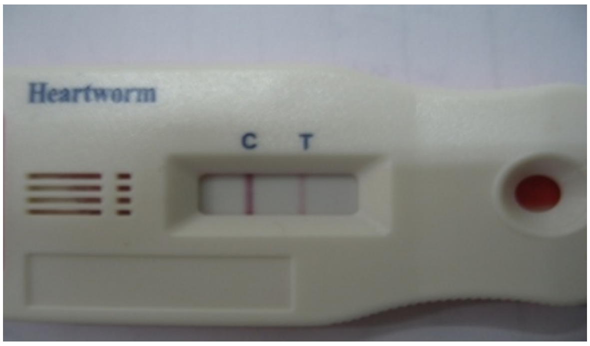


Figure2: Heartworm kit (positive immunochromatography assay)



Figure3: Electrocardiogram of the affected dog heart

Discussion

The present record confirmed the efficacy of ivermectin with levamisol and aspirin in the treatment of adult heartworm infection in a dog. It may be supposed that adult *D. immitis* was killed based because no further microfilaria were seen up to 6 months after the end of the treatment. Disease severity is classified on the basis of clinical signs, history, findings at physical examination, results of laboratory tests, and evaluation of radiographs. Four classes of heartworm disease have been established: subclinical or mildly clinical disease (class 1); moderate disease (class 2); severe disease (class 3); and caval syndrome, a very severe form of the disease (class 4). The treatment protocol in this study followed the producer's recommendations for therapy of moderate (class 2) heartworm disease. It is difficult to define the clinical signs that should be attributed to *D. immitis* in this dog. Coughing, dyspnea and limb edema found before the anti-filarial treatment recurred several weeks after treatment. The restlessness, respiratory distress and other clinical signs were not observed in our case. Thoracic radiography and electrocardiogram were done to characterize the degree of cardiopulmonary compromise and the severity of heartworm disease. The affected dog was more out door, and was thereby exposed to infectious agents. Therapy of *canine dirofilariosis* is important in order to decrease the risk of infection of other dogs and humans. Anaphylactoid reaction has been reported in a heartworm-infected dog undergoing lung lobectomy (Carter *et al.,* 2011). Previous reports of treatment and clinical management of *D. immitis* infections are few. It was suggested that doxycycline could impaire *D. immitis* transmission due to the reduction of microfilaria and also endosymbiotic bacteria in the larvae turning them incapable of completing development once they infect a new host (Rossi *et al.,* 2010). Treatment with ivermectin (6 µg/kg PO weekly) combined with doxycycline (10 mg/kg/day orally from Weeks 0-6, 10-12, 16-18, 22-26 and 28-34) resulted in a significantly faster decrease of circulating microfilaria and higher adulticidal activity compared with either ivermectin or doxycycline alone (Bazzocchi *et al.,* 2008). The covered-rod silicone implant containing 7.3 mg of ivermectin was 100% effective in preventing experimental infection with *D. immitis* larvae and had been resulted in negative results for heartworm antigen (Cunningham *et al.,* 2006). Oral diethylcarbamazine at 5.5 mg/kg daily for 1 month was used in Nigeria (Kamalu, 1991). Melarsomine (2.5 mg/kg) followed later by ivermectin (50 µg/kg) were used for treatment of a dog in Italy (Tarello, 2002). An ivermectin/pyrantel chewable formulation was found to be beneficial in the prevention of *D. repens* infection (Pollono *et al*., 1998). Otranto *et al.,* (2010) demonstrated that imidaclopride 10% and permethrin 50% preventive treatment against arthropods protects autochthonous and naive beagle dogs against canine vector-borne pathogens. It was suggested that a combination of doxycycline (10 mg/kg/sid for 30 days) and ivermectin-pyrantel (6g/kg of ivermectin +5mg/kg of pyrantel every 15 days for 180 days) was adulticide in dogs with *D. immitis*. Of 7 dogs that were positive for visualization

of parasites at echocardiography, 6 (85.7%) became negative by day 300. Treatment was well-tolerated by all dogs (Grandi *et al.,* 2010). Microfilaricidal effects of plant extracts (the aqueous extracts of Zingiber officinale) against *Dirofilaria immitis* has been suggested also (Merawin *et al.,* 2010). Imidacloprid plus moxidectin topical solution were effective for the prevention of heartworm disease in dogs (Arther *et al.,* 2005).

In the present case, therapy of ivermectin combined with levamisol and aspirin was effective to clear the *D. immitis* infection. Although ivermectin and levamisol are commonly used for the treatment of other helminthes infections in dogs, therapy with these drugs for *D. immitis* infestaion has not been approved for this specific indication. The initial treatment resulted in lowering the number of microfilaria nearly to 90% of their pre-treatment number, and the second treatment eliminated all remaining circulating microfilaria.

Melarsomine hydrochloride is a trivalent arsenical compound used as an adulticide for the treatment of canine heartworm disease. Doramectin has a prolonged activity and plasma half life, in comparison to ivermectin, which is commonly used as a microfilariacidal in dogs with heartworm disease (Greene, 2006, Knight, 1995). Doramectin has been shown to be safe in bitches treated with higher doses of 1 mg/kg (Schnieder *et al.,* 1996). Ivermectin is generally used against *D. immitis* at 0.05 mg/kg (Knight, 1995). Ivermectin was administered at dose 440 µg/kg in the present case.

In order to increase the acceptance of prophylaxis and reduce the likelihood of a false conclusion of prophylactic failure, education of dog owners should focus on the need for an appropriately timed annual heartworm test and the importance of administering the last dose of monthly heartworm preventative after the last possible day of potential transmission (Rohrbach *et al.,* 2011). In conclusion, the combination of ivermectin with levamisol and aspirin has been shown to be effective in clearing of *D. immitis* infection in dogs.

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عفونت ناشی از *دیروفیلاریا ایمیتیس* در یک قلاده سگ داشهوند: تشخیص و درمان

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چکیده

دیروفیلاریا ایمیتیس یک میکروفیلر است که فرم بالغ آن به طور معمول در بطن راست قلب و سرخرگ های تنفسی سگ ها زندگی می کند و می تواند موجب بیماری قلبی در سگ ها گردد. درمان دیروفیلاریوز سگ سانان ناشی از *دیروفیلاریا ایمیتیس* برای سگ هایی که از علائم بالینی این بیماری رنج می برند (نظیر سرفه مزمن) توصیه می شود. در این مقاله، یافته های تشخیصی و درمانی در یک قلاده سگ مبتلا به عفونت ناشی از *دیروفیلاریا ایمیتیس*، در منطقه اهواز، واقع در جنوب غرب ایران توصیف می شود. سگ دچار سرفه، تاکی پنه، تنفس صدادار، له له زدن، ادم اندام خلفی، ضعف و عدم تحمل تمرین، به هنگام مراجعه بود. سمع قلب یک مرمر سیستولیک درجه ۳/۶ را در سمت چپ نوک قلب نشان داد. گسترش های خونی حاوی میکروفیلر رنگ آمیزی شده با گیمسا، از نظر مورفولوژیکی *دیروفیلاریا ایمیتیس* تشخیص داده شدند. در CBC، لکوگرام آماسی و آنمی خفیف آشکار شد. درمان ترکیبی با داروهای آیورمکتین (۴۴۰ میکروگرم/کیلوگرم و به صورت تک دوز)، لوامیزول (۱۰ میلی گرم/کیلوگرم، روزانه و به مدت ۱۰ روز) و آسپیرین (۱۰ میلی گرم/کیلوگرم، هر ۱۲ ساعت و به مدت ۱۰ روز) ، در طی ۲ مرحله، در بر طرف شدن عفونت در یک قلاده سگ داشهوند، موثر بودند. تعداد میکروفیلرهای خون از ۵۰±۱۲۵۰ (قبل از درمان) به ۱۰±۱۵۰رسیده بود (متعاقب اولین مرحله درمانی، بعد از ۱۰ روز) و یک روز بعد از دومین مرحله درمانی، به صفر رسید. حیوان از نظر میکروفیلر *دیروفیلاریا ایمیتیس*، در طی یک دوره ۱۸۰ روزه منفی بود. این مطالعه کارایی آیورمکتین همراه با لوامیزول و آسپیرین را در درمان عفونت ناشی از کرم بالغ در سگ تایید می کند.

واژگان کلیدی: *دیروفیلاریا ایمیتیس*، سگ، آیورمکتین، لوامیزول، آسپیرین